Relationships Between Perinatal Interventions, Maternal-Infant Microbiomes, and Neonatal Outcomes

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KEYWORDS

• Microbiome • Perinatal • Pregnancy • Preterm birth • Prematurity • Neonate

KEY POINTS

- Premature neonates have a delay in the colonization of "healthy" commensal bacteria and a propensity toward harboring pathogenic bacteria, an attribute that may be a key etiology for the premature neonate's increased susceptibility to develop necrotizing enterocolitis or other infections.
- Mode of delivery does not seem to substantially alter the infant microbiome. Instead, only formula feeding and maternal diet have lasting impacts on the infant microbiome.
- The fetus does not lie in a sterile environment. It is likely that in utero exposure to microbes and/or a microbe's free DNA leads to fetal immune system priming and regulation.
- The maternal diet is a potent modifier of both the mother's and the infant's microbiome. Further studies are needed to evaluate the effects of the maternal diet on the breast milk microbiome.
- Dysbiosis of the maternal microbiome is currently a leading hypothesis underlying the etiology of preterm birth. Therefore, further studies evaluating the microbiome can help elucidate potential treatments for preventing preterm birth—the leading cause of death throughout the world in children under 5 years of age.

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INTRODUCTION

The human body is host to a diverse array of largely commensal bacteria, which collectively across all body niches comprise an individual's personal microbiome. The Human Microbiome Project, completed in 2012, sought to define reference "healthy" microbiomes by evaluating and characterizing the microbiome across multiple body sites in healthy individuals of different races and ethnicities in the United States. Overall, this robust, multicenter study found that niche specificity, bacterial diversity, and microbial gene carriage patterns far surpassed what was previously thought.[1–4] Importantly, commensal microbiota are more than simple bystanders because their presence and unique metabolic processes are essential components of our own physiology. Moreover, it is thought that the nature, state, and composition of the microbiome are related to (and likely contribute to) the development of several common human diseases. Dysbiosis of the human microbiome, defined as an aberrant microbial community, has been associated with the development of diabetes,[5–8] inflammatory bowel disease,[9–13] obesity,[14,15] metabolic syndrome,[16] and autoimmune disorders,[15,17,18–29] although causation has yet to be established.

In keeping with the developmental origins of health and disease hypothesis,[30–37] it is thought that the role of the microbiome in disease pathogenesis likely initiates in early life during key developmental windows, predisposing an individual to develop disease later in life when and if exposed to the right environmental triggers. Mice raised in the relative or complete absence of bacteria (gnotobiotic and germ-free mice) have immune deficits that cannot be restored completely unless the infant and mother are exposed to bacteria in pregnancy and early life.[38,39] For these reasons, understanding when and how the neonatal microbiome is first established, how it develops in the immediate postnatal period, and what external factors (eg, mode of delivery and breastfeeding) modify its trajectory has been a recent focus of the field.

Recent literature surrounding the perinatal microbiome has seen increased attention and focus. This article seeks to consolidate and evaluate the medical literature assessing common perinatal interventions, their effects on the infant microbiome, and their potential benefit to neonatal outcomes. First, pregnancy and the potential contribution of the maternal microbiome to preterm risk as well as the neonate's microbiome are discussed. Second, common perinatal interventions are explored, such as intrapartum antibiotic prophylaxis, mode of delivery, timing of delivery (premature vs term), hospitalization, and use of probiotics in both the mother and neonate. Finally, breastfeeding versus formula feeding is discussed and the impact each may have on neonatal outcomes.

PERINATAL AND POSTNATAL INTERVENTIONS AND THEIR IMPACT ON THE NEONATAL MICROBIOME

Although many perinatal interventions occur daily among the more than 4 million US births annually, including the use of probiotics or intrapartum antibiotic prophylaxis for group B streptococcus, and may seem relatively benign, the broad-reaching and longer-term impacts are unknown (**Fig. 1**). By contrast, broad-spectrum microbial interventions or manipulations have been studied a bit deeper and there is a bit more known about their impact on microbial communities, their structure, and their function. This article discusses the impact of preterm birth, common perinatal interventions, their influence on the fetal and neonatal microbiome, and the potential short-term and long-term neonatal outcomes.

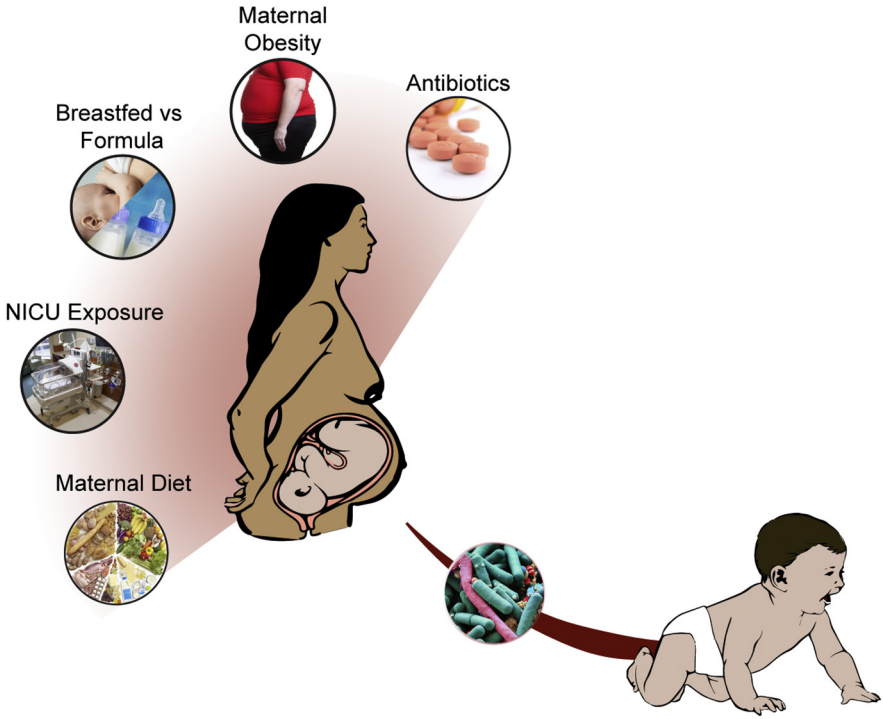

Fig. 1. Potential influences of the developing microbiome during pregnancy and early neonatal and infant life.

Preterm Delivery

Prematurity and the reasons leading to a preterm birth have lasting effects on both the neonatal microbiome and both the short-term and long-term outcomes in those neonates compared with delivery at term. As discussed previously, the maternal microbiome is essential in immune system priming of the fetus. Premature neonates are at higher risk for infection and intestinal problems, among other illnesses, owing to the lack of sufficient development of host tissues and immaturity of immune regulation at birth. Further issues may relate to a lack of time for the full effects of the in utero interactions with the maternal microbiome and/or a difference in the premature neonate's microbiome compared with the term neonate.

Thus, one key question to evaluate is, Does the microbiome differ between neonates born at term compared with those born preterm? One group of investigators from Spain evaluated 21 premature neonates' intestinal microbiota during the first 3 months of life and compared them to term, exclusively breastfed, vaginally delivered neonates. Premature neonates had increased levels of facultative anaerobic microorganisms and decreased levels of strict anaerobes, such as *Bifidobacterium*, *Bacteroides*, and *Atopobium*.[40] It is difficult, however, to assess if the changes they found are due to varying levels of gut maturity, lack of exclusive human milk feeding (all preterm infants included in this study received mixed feeding), or other associations with hospitalization and/or premature birth itself, for example, antibiotics. Furthering the idea that the microbiome is different among premature neonates compared with term neonates, other investigators have shown that

very-low-birthweight neonates have decreased diversity of their microbiota, which may be due to living in a hospital environment itself.[24,41–43]

Not only do premature neonates have a delay in the colonization of "healthy" commensal bacteria, such as *Bifidobacterium*, but also the premature neonate's microbiome contains higher quantities of pathogenic bacteria and readily loses the richness and abundance first seen at birth. *Klebsiella*, *Weissella*, *Clostridium, Enterobacteriaceae, Enterococcaceae, Streptococcaceae*, and *Staphylococcaceae* have all been found more commonly in premature neonates' microbiota than in neonates born at term.[44] Concurrent with these results, other investigators found increased levels of *K pneumonia* in the preterm infant microbiota, and *C difficile* was detected exclusively in the preterm infants.[40]

Thus, premature neonates are more prone to foster and harbor pathogenic bacteria rather than beneficial commensals, and the diversity and richness of their microbial communities first seen at birth simplifies days to weeks later and after periods of often intense interventions and isolation as well as antimicrobial therapy. This characteristic of prematurity (or its necessary interventions) may be one key reason why this age group has a higher likelihood of necrotizing enterocolitis (NEC) and other infectious maladies than term neonates.

The microbiome of preterm infants (eg, gestational age of 23–30 weeks) varies during the initial weeks of life, with dominance by *Escherichia*, *Klebsiella*, *Enterococcus*, and *Staphylococcus* dominant in the gut.[45] The presence of certain taxa do correlate, however, with increased gestational and postnatal age, for instance *Bifidobacterium.*[46] Colonization by *Bifidobacterium* is during the initial weeks of life in preterm infants is associated with protection from NEC[47] and late-onset sepsis.[48] Additional research is needed, however, to determine is this association is causal to the prevention of these diseases (eg, promotes gut maturation) or rather that colonization by *Bifidobacterium* simply reflects a more mature gut. Nonetheless, the potential to modify the preterm infant gut microbiome with probiotics is an area of active investigation. The most widely used probiotics in preterm infants are single or combination products consisting of *Lactobacillus* and *Bifidobacterium*. Overall, trials and meta-analyses in this area have shown conflicting findings in terms of NEC and sepsis diagnosis. Importantly, the receipt of probiotics results in shifts to both the gut microbiome and metabolome, with *Bifidobacterium* (but not *Lactobacillus*) able to colonize the gut of preterm infants long-term (even after discharge from the neonatal intensive care unit [NICU]).[49]

Mode of Delivery

Reported use of cesarean deliveries has been documented as early as the 1500s although its modern usage was not pioneered and promulgated until the mid-twentieth century after the discovery of penicillin.[50] Although 40 years ago, 1 in 20 births were delivered by cesarean, to date, that figure has climbed to nearly 1 in 3 in the United States. Obstetric guidelines put forth by the *American Journal of Obstetrics and Gynecology* (*AJOG*) outline specific indications for cesarean deliveries to ensure the health of the mother and her infant.[51,52] Although the risks to the mother in the immediate postoperative period and in future pregnancies are well documented, the long-term impact of a cesarean delivery on infant health and disease is not well understood. Numerous epidemiologic studies have inconsistently linked cesarean delivery with increased risk of allergy, metabolic syndrome, and obesity later in life,[53,54] although given their likely multifactorial and heterogeneous nature, it has been difficult to discern correlation versus causation.

The reported impact of cesarean delivery on the infant microbiome has been touted as the missing link between cesarean and future disease burden, resulting from a lack

of exposure to the microbial inhabitants of the maternal vagina. As such, investigators have already begun piloting vaginal swabbing of cesarean-born infants as means of correcting microbial community deficits. It is unclear, however, if these efforts are as yet justifiable without clear evidence of mechanism or direct benefit in relevant animal models.[53] Furthermore, the data supporting an association between cesarean delivery and an altered infant microbiome may be confounded by several clinical confounders, including prematurity, antibiotic usage, and maternal diabetes status, among others. In a large clinical cohort of longitudinally sampled mothers and infants, the authors found that the infant microbiome at 6 weeks of age did not vary by virtue of mode of delivery when controlling for various clinical factors. Only formula feeding and maternal diet seemed to have a lasting impact on the infant microbiome at this age. Therefore, although the question of whether or not cesarean delivery has a substantial long-term impact on the infant microbiome and ultimately in disease pathogenesis is uncertain, it nevertheless remains advisable to limit the use of cesarean deliveries by adhering to the official guidelines put forth by *AJOG* and other equivalent professional societies.

HUMAN BREASTMILK AND FORMULA FEEDING

The capacity to exclusive breastfeed or formula feed can have extensive effects on the neonatal microbiome. Breast milk and formula contain different bioactive components. For instance, formula contains macronutrients, vitamins, and a few oligosaccharides but is absent of the highly diverse human milk oligosaccharides (HMOs). Breast milk, however, contains macronutrients, vitamins, numerous HMOs and other oligosaccharides, growth factors, immune cells, immunoglobulins, hormones, cytokines, and a microbiome. The question becomes, Which components of human milk alter the neonatal enteric microbiome?

At the end of the nineteenth century, the overall infant mortality in the first year of life was as high as 30%. Medical providers noticed that breastfed infants had a higher chance of survival and lower incidence of infectious diarrhea than formula-fed infants.[55] Researchers began looking at what in breast milk may be protecting these neonates from increased mortality. Investigators soon found that the feces of breastfed infants contained different bacteria from those of the bottle-fed cohort.[56] In 1926, Schonfeld[57] published findings of a growth-promoting factor for *Bifidobacterium bifidus,* a protective commensal, contained in breast milk. This “bifidus factor” was later confirmed to be HMOs.[58–62] Now, more than 100 different HMOs have been identified, and not every woman has the same production of HMOs.

HMOs, besides being the bifidus factor, have numerous health benefits for the neonate. Because HMOs are resistant to the acidity of the infant stomach, they reach the distal small intestine and colon intact and at high concentrations. *Bifidobacterium longum* subsp *infantis (B infantis)* grows especially well when HMOs are present in the neonatal intestine. The proliferation of *B infantis* helps prevent pathogenic bacteria replicating as they compete for a limited nutrient supply. Also, *B infantis* is known to produce short-chain fatty acids that favor the growth of commensals in niches that might otherwise be colonized by potentially pathogenic bacteria.[55,63] Moreover, HMOs prevent the adhesion of viral, bacterial, and protozoan pathogens from attaching to the enteric epithelium, and, thus, prevent enteric infections in the neonate.[64,65] Another protective attribute of HMOs is that they serve as decoy attachment receptors for pathogens, which can reduce infection rates.[66,67] In fact, HMOs may even block HIV entry via preventing the attachment of the virus to its entry receptor DC-SIGN, and it may explain why mother-to-child transmission of HIV through breastfeeding is

inefficient, with up to 90% of infants not acquiring infections despite continuous exposure to the virus through the breastmilk.[68]

A MOMS MATERNAL DIET, HER MICROBIOME, AND ITS POTENTIAL IMPACT ON LONG-TERM INFANT HEALTH

The prevailing dogma indicates that the neonate is born sterile and only after delivery is the neonate populated by bacteria. Under this belief, the French pediatrician Henry Tissier professed in 1900, "The fetus lies in a sterile environment."[69] Both historical evidence and more contemporary evidence, however, have challenged the notion of a completely sterile intrauterine environment. In 1982, bacteria were found present in the placenta, which began the pursuit of other researchers to determine if this was accurate.[70] More recently, numerous groups using both conventional culturing techniques and contemporary 16S rRNA gene and/or metagenomic sequencing, have found evidence of bacteria in association with presumed "sterile" tissues of healthy term pregnancies, such as the placenta and amniotic fluid.[71–81] Therefore, not only may the developing fetus be exposed to bacteria earlier than believed but also detection of microbial DNA has been well established in neonates at birth as well as fetuses and placentae prior to birth. Presumably, these microbes originate from the mother, although the route through which these organisms can enter the intrauterine space is not clearly established.[82,83] The authors and other investigators leading this field, however, remain uncertain if the organisms are viable or if free DNA is being detected. Still, the observation of a nonsterile intrauterine environment by metagenomic and other measures indicates that the contribution of the maternal microbiome in pregnancy may be as important to the neonatal microbiome as the immediate postnatal period.

Evidence of the Maternal Microbiome Influencing Neonatal Development

The role of maternal exposures and the maternal microbiome in the community and functional establishment of the infant microbiome may also influence immune repertoire and functional development. To test this hypothesis, one study evaluated the impact of the maternal microbiome on the intestinal immune system development of the offspring. Gomez de Aguero and colleagues[84] devised an experiment in which germ-free pregnant mice were transiently colonized with a genetically engineered form of *Escherichia coli*, which does not persist in the murine intestine. The pregnant mice that were transiently colonized gave birth to pups who had increased intestinal innate lymphoid cells and mononuclear cells compared with controls. In addition, these same researchers further looked into the effects of maternal antibodies and maternal microbial molecular transfer on the priming of the fetal immune system. They found that the maternal microbiome and maternal antibodies promote transfer of noninfective microbial molecules to the fetus. These microbial molecules are believed to prime the neonatal innate immune system and prepare the fetus for the postnatal inundation of microbes that eventually colonize the neonatal intestine.[84] Thus, microbes are essential in priming the fetal immune system and preparing the fetus against the plethora of pathogens it will soon encounter after birth (**Fig. 2**).

The Impact of the Maternal Diet and Health on the Early Neonatal Microbiome

The maternal microbiome fluctuates throughout gestation and is associated with obesity, altered caloric density, and content of diet, and comorbidities, such as gestational diabetes. Emerging evidence suggests that many of these factors are associated with differences in the early neonatal microbiome, suggesting that the state of

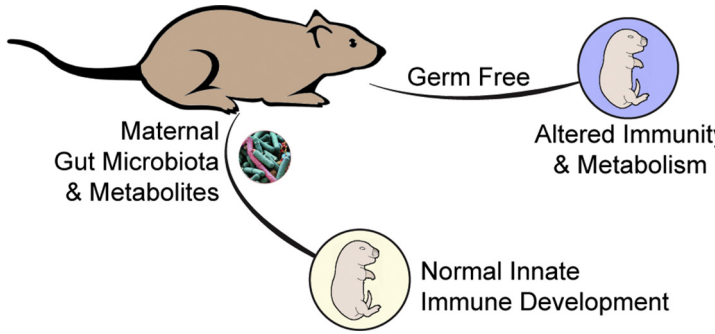

Fig. 2. Evidence from rodent studies suggesting the importance of microbes during gestational development for metabolic and immune health among offspring.

the maternal microbiome in pregnancy can have a considerable impact on what is transmitted to the neonate and how a microbiome ultimately develops. Diet is a potent modifying factor of the adult gut microbiome and consequently, in both animal models and human cohorts, the composition of the maternal diet in pregnancy is associated with distinct changes in the immediate neonatal gut microbiome as well.[83,85–87] In the Japanese macaque, a neonate born to a mother consuming a relatively high-fat diet in pregnancy was associated with a depletion of commensal species like *Campylobacter*, whereas in humans a maternal high-fat diet was associated with lower levels of *Bacteroides* species.[83,87] Work in animal models has demonstrated that commensal enteric species, in particular *Bacteroides*, are vital for normal gut immune development; therefore, lacking these beneficial microbes during this early developmental window is hypothesized to have a lasting effect on the neonate, ultimately predisposing the infant to atopy and other autoimmune disorders later on in life.[88–90]

The effect of maternal diet seems to extend beyond gestation. Research has shown that a high-fat diet leads to increased milk fat concentration and content compared with a high-carbohydrate diet.[91] No differences in milk production or quantity of milk, however, were observed. Therefore, neonates consuming breast milk from mothers with a high-fat diet consume higher energy intake, which can have potential effects on the development of their microbiome. Although it has not been studied, differences in the properties of the breastmilk likely affect which bacteria flourish in the neonatal microbiome, but further studies are needed to confirm this hypothesis. Along these same lines, the maternal diet may be associated with alterations in the breast milk microbiome. Unfortunately, there currently are no studies published evaluating this association. Investigations are currently exploring this hypothesis and will help understand any substantial impact the diet has on the breastmilk microbiome.

Maternal chronic conditions, such as gestational diabetes, overweight status, and obesity, are now known to have associated changes in the maternal microbiome. Bassols and colleagues[92] showed that women with gestational diabetes have a distinct placental microbiota profile, which includes a lower abundance of *Acinetobacter*, which was associated with higher glucose concentrations and a more proinflammatory maternal phenotype. In another study, Gomez-Arango and colleagues[93] evaluated the relationship between the enteric microbiome and metabolic hormones in overweight and obese pregnant women. Elevations of specific hormones, such as insulin, adipokine, and glucose-dependent insulinotropic polypeptide, were associated with elevations or reductions of specific bacteria. These results suggest that the enteric microbiome may have the potential to influence metabolism of the pregnant

woman. The impact that these chronic conditions have on the fetus and neonate has not been extensively studied and is still open for investigation.

Antibiotic Administration

As discussed previously, the maternal microbiome potentially helps maintain pregnancy. Antibiotics disrupt and alter the microbiome. Thus, it is a natural progression of this thought that antibiotics and their effects on the microbiome can affect not only the mother but also the fetus. Antibiotics account for a majority of prescribed medications during pregnancy. One large Danish study showed that 51% of women had received 3 or more courses of antibiotics in the 4 years before, during, and after pregnancy.[94] In another study, maternal antibiotic administration was associated with a 30% increased risk of the offspring developing asthma.[94] Also, antibiotic usage in the pregnant woman has been linked to higher rates of neonatal illnesses, such as NEC, and increased rates of cerebral palsy and developmental delay.[95,96]

Further investigating the role of antibiotics on the maternal microbiome and neonatal outcomes, a murine model was devised. Pregnant, nonobese diabetic mice were given antibiotics. The offspring were observed to have immunologic changes in their intestines compared with those that did not receive antibiotics prenatally.[97] Furthermore, in humans, antibiotics during pregnancy alter the vaginal microbiome, which then lead to changes in the colonization and development of the neonatal microbiome[98] as well as an association with increased childhood obesity[99] and asthma.[94,100,101]

A systematic review published in 2013 showed that prophylactic antibiotics during the second or third trimesters in mothers with intact membranes do not decrease adverse outcomes and morbidity in pregnancy.[102] Also, even short-term antibiotic administration can have long-standing effects on the microbiome with possible associated changes in the immune system. In another study of 198 healthy term infants, maternal intrapartum antibiotic prophylaxis and birth method were documented. The infant gut microbiota was significantly different with intrapartum antibiotic prophylaxis exposure with persistence of the differences up to 12 months of age, with the findings found in both caesarean and vaginal deliveries.[103] An increase in pathogenic bacteria, such as *Enterococcus* and *Clostridium*, were over-represented at 3 months after the maternal intrapartum antibiotic prophylaxis. These findings support that antibiotics, even if for a short course, such as with intrapartum antibiotic prophylaxis, do have long-lasting effects on not only on the mother but also the neonate.

What, however, is the impact of antibiotics given directly to the neonates directly after birth, such as for early onset sepsis, on the neonatal microbiome? Premature neonates almost universally receive antibiotics at some point in their stay in the NICU, many in the first days of life. Gibson and colleagues[104] evaluated the impact of neonatal antibiotics on the development of antibiotic resistance, species diversity, and the prevalence of pathogenic bacteria predominating the neonatal microbiome. They found that antibiotics significantly decreased species richness and diversity in the intestinal microbiome.[104] In addition, multidrug-resistant bacterial members of the genera *Escherichia*, *Klebsiella*, and *Enterobacter* predominated the premature neonatal gastrointestinal microbiota.[104]

Brooks and colleagues[105] found in a separate study that bacteria from the neonate's surroundings in the NICU may be directly inoculated into the neonate and be the source of the pathogenic bacteria. For example, one infant had *K pneumonia* detected in the room on day of life 3, and it was then detected in the neonate's gut on day of life 9.[105] *Staphylococcus epidermidis*, *K pneumoniae*, *Bacteroides fragilis*, and *E coli* were found widely distributed throughout the rooms of the neonates, and they are all

well-known pathogenic gastrointestinal colonizers.[105] Does the premature gut have a predisposition for these pathogenic colonizers due to the lack of time in utero, which helps facilitate the gut immune priming and development? Could early use of antibiotics predispose these neonates to these pathogens? Or, is it just simply living in the hospital environment? Likely, it is a combination of these factors, but further research is currently exploring these and other questions. Regardless, antibiotics given intrapartum as well as postpartum have direct effects on the neonatal microbiome.

THE MICROBIOME AND PRETERM BIRTH: FRIEND OR FOE?

Preterm birth is the leading cause of death among children under the age of 5 years old throughout the world, although its etiology is poorly understood and effective prevention and treatment options are lacking.[106] Although heterogeneous in nature, preterm birth is hypothesized to have an infectious etiology. A pathogenic organism, however, has yet to be attributed to preterm birth whereas antibiotic usage for a presumptive infection has not been shown to provide benefit.[107,108] The emergence of microbiome science applied to human health has led to the hypothesis that community level changes to the maternal microbiome is contributory to premature labor, rather than by infection of a specific microbe.[109] As such, efforts to characterize the "healthy" maternal microbiome of the vagina and other body sites has been prioritized, with the intent of identifying patterns of deviation associated with preterm birth risk.

During pregnancy, a woman's body undergoes both physical and hormonal changes across nearly every organ system to support fetal development, ready for parturition, and prepare for lactation in the postnatal period. Similarly, the maternal microbiome undergoes complementary rearrangements that are believed beneficial (a more thorough review of these changes can be found by Chu and colleagues[110]). The most notable of these changes with respect to preterm birth risk is the observation that in healthy pregnancies, the overall diversity of the vaginal microbiome tends to decrease into the third trimester, with *Lactobacillus* species tending to become the dominant member.[111] Within the vaginal milieu, lactobacillus species are believed to provide a natural defense against pathogenic overgrow within the vaginal canal by maintaining a low vaginal pH and may potentially explain the longstanding association of bacterial vaginosis with preterm birth.[112–115]

For these reasons, aberrant microbial communities, otherwise known as dysbiosis, in the vaginal microbiome has been hypothesized to contribute to premature labor, potentially by promoting proinflammatory cytokines that can be stimulated by such a derangement of the microbial milieu. There are conflicting data, however, on whether the vaginal microbiome has a characteristic profile that reliably predicts preterm birth.[116,117] Romero and colleagues[117] reported that the bacterial composition and abundance did not differ between mothers who delivered preterm compared with those who delivered at term whereas, conversely, DiGiulio and colleagues[116] reported that reduced *Lactobacillus* and increased *Gardnerella* or *Ureaplasma* were associated with increased risk of preterm birth. The discrepancy between these studies may be in part confounded by the known variation of the vaginal microbiome throughout pregnancy, which is characterized by enrichment of *Lactobacillus* with increasing gestational age, reduced overall richness and diversity, and greater overall stability.[116,117] Alternatively, ethnic and racial differences between the 2 cohorts may account for the differences seen between these studies, but this has yet to be accounted for in the current literature.

The uncertain association between the vaginal microbiome and preterm birth has spurred investigation into the microbial communities of other body sites, including

the mouth, the gut, the placenta, and the intrauterine environment. Within the gut, Shiozaki and colleagues[118] found that the fecal microbiota had significantly higher levels of *Clostridium* and reduced levels of *Bacteroides* in women who had a preterm birth, although the impact of such changes to preterm labor is unknown.[118] Other intriguing studies have shown that the placental microbiome, which most closely resembles the oral microbiome, is altered in cases of preterm birth, whereas amniotic fluid collected in women who have preterm birth harbor bacteria from the oral cavity. These observations have been intriguing considering that periodontal disease is associated with a 7-fold increased risk of preterm birth.[119] Periodontal disease may lead to hematogenous spread of bacterial pathogens to the placenta and the fetus, disrupting the placental microbiome and ultimately leading to premature labor, although further studies in controlled animal models are needed evaluate this potential mechanism.

A microbiome-centric perspective has the potential to innovate the way in which interventions for preterm birth prevention are developed and administered. Traditionally, for microbe-related disorders, antibiotic regimens are given to kill the pathogenic organisms. In the context of preterm birth, however, such efforts have shown ineffective or even increase risk of preterm birth. Treatments targeting bacterial vaginosis during pregnancy did not prevent preterm birth in multiple studies and increased risk in others.[120–122] A 2012 Cochrane review demonstrated that treatment can eradicate

Table 1
Table of key topics in relationship to neonatal microbiome research and key associated articles

Topic	Key References Pertaining to Topic
Preterm delivery and effects on neonatal microbiome	Arboleya et al,[40] 2012; Schwiertz et al,[24] 2003; Magne et al,[41] 2006; Roudière et al,[42] 2009; Rougé et al,[43] 2010; Morowitz et al,[44] 2011; Stewart et al,[45] 2017; Butel et al,[46] 2007; Stewart et al,[47] 2016; Stewart et al,[48] 2017; Abdulkadir et al,[49] 2016
Mode of delivery and neonatal microbiome	Boley et al,[50] 1991; Aagaard et al,[53] 2016; Yuan et al,[54] 2016; American College of Obstetricians and Gynecologists,[51,52] 2014
Breastmilk and formula feeding and neonatal microbiome	Bode,[55] 2012; Gauhe et al,[58] 1954; Gyorgy et al,[59–61] 1954; Rose et al,[62] 1954; Gibson & Wang,[63] 1994; Kunz et al,[64] 2000; Newburg et al,[65] 2005; Simon et al,[66] 1997; Gustafsson et al,[67] 2006
Maternal diet and neonatal microbiome	Ma et al,[83] 2014; David et al,[85] 2014; Gohir et al,[86] 2015; Chu et al,[87] 2016; Troy & Kasper,[88] 2010; Round & Mazmanian,[89] 2010; Mazmanian et al,[90] 2005; Mohammad et al,[91] 2009; Bassols et al,[92] 2016; Gomez-Arango et al,[93] 2016
Antibiotics and neonatal microbiome	Stokholm et al,[94] 2014; Kenyon et al,[95] 2001; Kenyon et al,[96] 2008; Tormo-Badia et al,[97] 2014; Stokholm et al,[98] 2014; Mueller et al,[99] 2015; Vidal et al,[100] 2013; Jepsen et al,[101] 2003; Flenady et al,[102] 2013; Azad et al,[103] 2016; Gibson et al,[104] 2016; Brooks et al,[105] 2014
Microbiome and birth associations	Brocklehurst et al,[107] 2013; Oliver & Lamont,[108] 2013; Baldwin et al,[109] 2015; Chu et al,[110] 2016; Aagaard et al,[111] 2012; Litich et al,[112] 2003; McDonald et al,[113] 1991; Romero et al,[114] 2002; Hay et al,[115] 1994; Offenbacher et al,[116] 1996; Nygren et al,[117] 2008; McDonald et al,[118] 2007; Thinkhamrop et al,[119] 2015; Schwiertz et al,[15] 2010; Koren et al,[16] 2012

bacterial vaginosis but does not have any significant impact on the prevention of preterm birth or preterm prelabor rupture of membranes. Therefore, it is not recommended to screen and treat all pregnant women with bacterial vaginosis to prevent preterm birth or preterm premature rupture of membranes.[107]

Ultimately, therapies aimed at preventing preterm birth may focus on maintaining healthy communities, rather than treating for specific pathogens. Before such treatment approaches are realized, however, additional work is needed to evaluate the impact of the maternal microbiome across different body niches, inclusive of the mouth, the vagina, and the intrauterine environment.

SUMMARY

Overall, perinatal interventions have significant impact on both the maternal and neonatal microbiomes (**Table 1** lists references per topic). Some key questions, however, are still left unanswered. Does dysbiosis of the enteric microbiome play a larger role in the development of preterm birth? If so, are there any therapies that can be targeted to ameliorate this dysbiosis and protect against preterm birth? There is limited research evaluating the impact of antenatal steroids on the fetal and/or neonatal microbiome. How do antenatal steroids affect the maternal, fetal, and neonatal microbiomes? Finally, it is known that microbial contact begins in utero, but is this true colonization or just transient seeding? Because it is virtually impossible to ascertain this fact in human experiments, future investigations in animal models are imperative to help determine if it is truly colonization or simply a transient seeding that has an impact on the fetus. These and other questions are now imperative for researchers to address, to evaluate and further the understanding of the microbiome, its development, and its potential interactions on the development of disease states in the host. Through this understanding, potential directed therapies can be initiated, which could lead to improvements in neonatal outcomes throughout the world.

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